Detoxification of Aflatoxin-Contaminated Corn

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ABSTRACT

Outbreaks of aflatoxin in corn occur sporadically in different sections of the United States. In recent years, the incidence has centered largely in the Southeastern States. When these outbreaks occur, some means of salvaging the contaminated corn must be considered. Numerous approaches have been suggested, largely falling in the broad categories of physical separation, chemical inactivation, and biological inactivation. Research conducted on peanut and cottonseed meals has provided a basis for the selection of methods to be applied to the detoxification of corn. From a review of past and recent literature, it appears that the most practical method for salvaging aflatoxin-contaminated corn is by ammoniation. Extensive work at NRRC resulted in development of a procedure whereby introduction of ammonia reduced aflatoxin in corn from in excess of 1,000 ppb to less than 10 ppb. Although toxicological studies have not yet been completed to qualify the process for FDA approval, indications are that the method is a viable one and should offer promise to farmers and others who may face financial disaster as a result of aflatoxin.

INTRODUCTION

Aflatoxins are secondary metabolites of two widely distributed toxin-producing fungi, Aspergillus flavus and Aspergillus parasiticus. Although aflatoxin is only one of the many metabolites produced by toxin-producing fungi, it is probably the most well-known and certainly the most studied. Since 1960, when aflatoxin in peanut meal was found to be the cause of the death of more than 100,000 turkey poults in England, extensive investigations have been conducted on the many ramifications of the problem. A CAST report (116) assesses the problems involving aflatoxin, as well as other mycotoxins that have been found to occur naturally in plant products, and gives a broad overview of past studies on aflatoxin production, biological effects, economic aspects, and control measures that offer possible solutions to agriculture.

Aflatoxin is found more commonly in some plant products than in others. Among the high-risk crops are peanuts and peanut meal, corn and corn meal, Brazil nuts, cottonseed and cottonseed meal, and copra. In general, the major food grains, i.e., wheat, soybeans, sorghum, barley, and oats, are relatively low aflatoxin risks. In grain, aflatoxin production was thought to be largely a storage problem (123, 234, 356, 357). However, studies by Anderson et al. (23) showed that aflatoxin was found in corn in the field at all stages of development, from the late milk stage until harvest. Insect damage was observed in 90 percent of the samples. Christensen et al. (126) pointed out that where developing ears are invaded by certain insects, they are likely to be invaded by A. flavus and aflatoxins may be produced. Such insect invasion is common in corn in the Southern United States, and aflatoxin commonly is present in corn at harvest in those areas, sometimes in large amounts.

The best approach to the aflatoxin problem is prevention, and enough is known about prevention to considerably reduce contamination. Guidelines for preventing mycotoxins in farm commodities were suggested by the U.S. Department of Agriculture some years ago (611, 612) and have been further elaborated by Goldblatt and Dollear (231) and Goldblatt (230). These guidelines dealt primarily with recommended practices for growing, harvesting, handling, and storage. However, aflatoxin production and contamination may occur despite the efforts directed at prevention. Alternative measures then must be taken so that the contaminated commodity might be rendered useful. Many ap-

proaches have been suggested, generally involving physical separation methods and inactivation by biological or chemical means.

Until the early 1970's, aflatoxin in corn was not recognized as a major problem. Rather, attention was confined mainly to contamination in peanuts and cottonseed and in their meals resulting from the extraction of the oil. Considerable work has been reported on the decontamination of aflatoxin in these commodities by the Southern Regional Research Center. This work has been reviewed extensively by Goldblatt (230) and Goldblatt and Dollear (232) and will not be discussed in this paper, which will be restricted to the decontamination of aflatoxin in corn. The research on decontamination of oilseeds containing aflatoxin contributed immensely to the corn investigations, providing an extensive background in methodology, technology, and selection of effective chemicals or biological agents for the destruction or degradation of aflatoxin.

Processes for detoxifying aflatoxin must take into consideration the usual nonhomogeneity of the contamination, that is, aflatoxin often is localized in only a small proportion of the contaminated product. This may prove an asset in some inactivation methods but poses a difficulty in others. Procedures that have been investigated for detoxifying corn can be divided into three categories: physical, biological, and chemical.

PHYSICAL SEPARATION Mechanical Separation

Physical separation techniques, such as sieving and electronic and hand sorting, have been investigated as possible means for segregating contaminated peanuts and other nut meats (437, 164). The need for both electronic sorting and hand picking is emphasized, because it is indicated by Dickens and Whitaker (164) that either method alone is inadequate.

The use of these techniques to segregate contaminated kernels of corn is much more difficult than for peanuts; in contrast to contaminated peanuts, which are usually damaged, shriveled, or discolored, contaminated corn kernels can appear sound, thus obviating any electronic sorting process.

Brekke et al. (84) found that physical separation methods were generally ineffective for lowering the aflatoxin content of naturally contaminated corn used in the experimental work. The corn lots tested differed in aflatoxin content (10 to 450 ppb B₁), geographic source, and content of broken corn-foreign material, and represented both white and yellow corn. Dry cleaning, wet cleaning, density separation, and preferential fragmentation of the grain were evaluated in laboratory tests. Aflatoxin was concentrated in broken corn-foreign material in only one of the ten lots tested.

^{&#}x27;The mention of firm names or trade products does not imply that they are endorsed by the U.S. Department of Agriculture over other firms or similar products not mentioned.

Hand-selected kernels that outwardly appeared sound and free of the bright greenish-yellow fluorescence associated with the presence of aflatoxin had the toxin in excess of current FDA guidelines (20 ppb) in six out of seven lots of corn. The hand-selected kernels contained about one-half to as little as one-tenth the level of aflatoxin in the unfractionated lot.

Huff (294) demonstrated a method for segregating aflatoxincontaminated corn from sound corn. Two lots of corn differing in degree of contamination (527 and 3,317 ppb) were segregated into buoyant and nonbuoyant fractions in water and three sucrose solutions (20, 30, and 40 percent). He found that aflatoxincontaminated corn was buoyant in these liquids and could be separated from the sound material, with a significant reduction in overall level of aflatoxin in the nonbuoyant corn. Although this procedure may be impractical at this time, it suggests that density differences between contaminated and sound grain could provide a basis for designing various segregation processes.

Milling

When corn containing aflatoxin was dry-milled, the contamination appeared to concentrate in the nonendosperm fractions. Brekke et al. (86) dry-milled three lots of corn (one yellow, two white) naturally contaminated with different levels of aflatoxin (13, 160, and 510 ppb of B₁) to determine distribution of the toxin among product fractions. Product yields and fat contents were fairly typical of those for normal dent corn. Aflatoxin level was always lowest in the grits and highest in the germ, hull, or degermer fines and varied with the contamination level of corn being milled. Proportion of aflatoxin B₁ (AFB₁) in the prime product mix (i.e., grits, low-fat meal, and low-fat flour) amounted to only 7 to 10 percent of total quantity of AFB1 in all products. Concentrations of AFB1 in degermer fines, germ, and hull exceeded that of the corn milled. The AFB₁ level in endosperm-derived products correlated with their fat content. Yield of prime product mix, based on all products recovered, varied between 49 and 60 percent in these

In the wet-milling of aflatoxin-contaminated corn, aflatoxin again was concentrated in the feed fractions. Yahl et al. (672) inoculated corn with spores of A. flavus and allowed incubation to proceed until mold growth was observed. This corn was found to contain 638 ppb of AFB₁. This sample and a naturally contaminated sample (120 ppb AFB₁) were steeped and wet-milled by a laboratory procedure. Aflatoxin was found primarily in steepwater (39-42) percent) and fiber (30-38 percent) with the remainder in gluten (14-17 percent) and germ (6-10 percent). Increases in concentrations of AFB1 in the fractions compared with the original corn were steepwater, 4- to 5-fold; fiber, 2.5- to 3-fold; gluten, 1- to 1.5-fold; and germ, 1-fold. The starch fractions had AFB₁ levels of 9.0 and 2.2 ppb, about 1 percent of the toxin originally in the corn. Analyses of 105 samples of commercial corn steepwater produced at six individual plants over a period of 1 to 3 months during new corn movement gave negative aflatoxin results in all cases. Assays of a number of production samples of starch, germ, gluten, and gluten feed were all negative. Analytical procedures gave sensitivities of 1 to 3 ppb of AFB₁. Both the dry- and wet-milling industries screen their incoming corn carefully to prevent aflatoxin-contaminated corn from entering their plants.

Heat

Christensen et al. (126) stated that aflatoxin usually endures for a long time. It is not totally destroyed or inactivated by temperatures up to or above that of boiling water or by any processing the grain undergoes during the preparation of feeds. Marth and Doyle (397) have reviewed the use of heat in reducing aflatoxin in oilseed meals. They cited the use of roasting as a method for reducing

aflatoxin by 45 to 70 percent, and also pointed out that the presence of moisture was necessary to effect greater reduction.

Conway et al. (141) at the Northern Regional Research Center demonstrated that application of the roasting process to corn could materially reduce aflatoxin in contaminated corn. Using commercial roasters, they reduced aflatoxin 40 to 80 percent by a single passage through a continuous roaster. This investigation on roasting contaminated corn that had been pretreated with ammonia resulted in reductions in aflatoxin as high as 90 percent. In these tests, the corn was tempered to 20 percent moisture with aqua ammonia to give 0.5% NH3 db, held for 3 hours, and then passed through the roaster. When the corn was retempered as described above and again passed through the roaster, further reduction in aflatoxin resulted. It appears that this procedure may be a simple and effective route to decontamination of corn. Further work should be conducted on this approach. Another possibility for using heat to decontaminate aflatoxin, particularly in peanut and cottonseed meal, is the application of extrusion processing, with or without chemical pretreatment. This research approach is being evaluated by workers at Texas A & M University.

Others

The use of irradiation to reduce aflatoxin in oilseed meals has been reported in reviews by Ciegler (131) and Marth and Doyle (397), but this approach is not considered practical at this time. The Southern Regional Research Center has studied the use of polar solvents or mixtures of them to reduce or eliminate aflatoxin in oilseeds. Goldblatt (230) has reviewed this approach and, although the methods appear to be effective in removing aflatoxin, they do affect the nutritive value of the extracted meal and are costly.

BIOLOGICAL INACTIVATION

Ciegler (131) and Marth and Doyle (397) have reviewed the application of microorganisms for the purpose of destroying or transforming aflatoxin B₁. Numerous investigators have provided information that indicates some reduction in AFB₁ can be attained through use of certain fungi, bacteria, and yeasts. None of the procedures have been conducted on a large scale, and to date, none have been reduced to practice. The biological approach does offer another possible solution to the problem of aflatoxin contamination, but much additional research is needed.

CHEMICAL TREATMENT

Inactivation of aflatoxin by chemical treatment appears to offer the most promising and feasible approach. Indications are that two points of the aflatoxin molecule are most susceptible to chemical attack: the internal ester of the coumarin moiety, and the double bond of the terminal furan, when it is present. The literature cites the application of many different chemicals to the treatment of peanut and cottonseed meals for the inactivation of aflatoxin. Included in the extensive listing are ozone (187, 173, 495), hydrogen peroxide (573), methylamine (173, 390), sodium hypochlorite (438), formaldehyde and calcium hydroxide (136), and ammonia.

Use of aqueous or gaseous ammonia with or without heat and pressure has received the most attention in the United States for detoxification of aflatoxin-contaminated cottonseed, peanut meals, and corn. Masri et al. (400) ammoniated contaminated peanut meal containing 709 ppb AFB₁, moistened to 9.6 percent and 14.6 percent at 200°F for 60 minutes at 20 psig anhydrous ammonia pressure and reduced the AFB₁ by 96.4 percent and 97.6 percent, respectively. Dollear et al. (173) obtained essentially the same results in a slightly modified process. In large-scale pilot plant runs. Gardner and his coworkers (223) determined optimum processing conditions to inactivate aflatoxins in lots of one or more tons of oilseed meals by ammoniation. Moisture levels were adjusted to 12.5 percent and temperatures to 235° to 250°F, and anhydrous

ammonia was injected to reach 45 to 50 psig. At the end of the run, monocalcium phosphate was added to absorb residual ammonia in the meal to give an odor-free product. Aflatoxin content was reduced from 121 ppb to less than 5 ppb.

A similar process utilizing ammonia, but without pressure or high heat, to detoxify corn has been developed on a large scale at the Northern Regional Research Center, Peoria, Illinois (85, 87, 90). The procedure, carried out at atmospheric pressure and ambient temperature, was conducted in a standard grain bin with a perforated floor, which had been made essentially airtight. Working with contaminated corn in lots as large as 1,000 bushels, the moisture content of the grain was adjusted to 15-22 percent, wet basis, with 18-19 percent being typical. After allowing the moisture of the corn to distribute and equilibrate (3 to 24 hours), ammonia was added in gaseous form to provide a level of 0.5 to 1.5 percent, dry basis. Through a recycling system connected to the bin, the ammonia-air mixture was recycled through the bed of corn for 24 hours, then held an additional 12-13 days to ensure detoxification of the grain. The corn is then dried to safe storage moisture. Treatment with ammonia of corn naturally contaminated with up to 1,000 ppb total aflatoxins reduced the contaminant to less than the current FDA guideline of 20 ppb.

Research was next directed toward evaluating the safety of the process to ascertain whether the reaction of ammonia, aflatoxin, and corn might result in a product with toxic properties. Brekke et al. (88) published the results of a study in which ammoniated corn was fed to rainbow trout, which are very susceptible to the carcinogenic effects of aflatoxin. The control corn contained a total of 180 ppb aflatoxin and trout fed at a level of 25 percent of the ration for 12 months developed a hepatoma incidence of 96-98 percent. The incidence was much lower (< 3 percent) in trout fed similarly contaminated corn that had been ammoniated. Jensen et al. (304) conducted four trials involving 356 pigs to evaluate acceptance and utilization of ammoniated corn. Corn, naturally contaminated with 36, 39, or 90 ppb of AFB₁ was ammoniated and the aflatoxin concentration reduced to a nondetectable level, as determined by chemical analysis. Ammoniated corn fed free choice with a supplement were consumed in lesser quantities than nonammoniated corns, with associated greater consumption of the supplement. Acceptance and utilization of mixtures of ground ammoniated corn and supplement, however, were equal to nonammoniated corn and supplement mixtures when water-extractable ammonia content of the corn dry matter was approximately 0.1 percent or less.

Hughes et al. (295) investigated the safety of feeding ammoniated corn to white leghorn laying breeders. Four types of corn were used: normal corn (control); ammoniated control corn; aflatoxin-contaminated corn (754 ppb); and ammoniated aflatoxin corn (resulting in an AFB1 level of 3.5 ppb). Corn made up 66.3 percent of the total diet, resulting in an aflatoxin level in the feed of 500 ppb and 2.3 ppb for the third and fourth treatments, respectively. The study consisted of two trials each with a duration of ten 28-day periods. White leghorn pullets were 26 weeks of age at the start of the first trial and 22 weeks of age at the start of the second trial. The birds receiving the ammoniated corn showed no deleterious effects on production, egg quality, reproduction, feed consumed per dozen eggs, or mortality rates. There was a trend for these birds, especially the aflatoxin-ammoniated group, to consume less feed and gain less body weight during the trial. However, as noted above, this did not cause any problems with other parameters. Thus, corn treated by the ammoniation process used in this study would be safe in white leghorn layer-breeder diets at levels ≤ 66.3 percent of the total diet. The presence of 500 ppb of AFB1 in the feed did not cause any serious problems. Differences were observed for each of the following parameters, but the differences were usually small and were significant in only one trial of the study: reduced egg white (second trial), reduced total mass of eggs produced (second trial), increased percentage of blood spots (second trial), reduced feed consumption per bird per day (second trial), and reduced day-old chicken weight (first trial).

Norred (447) studied the effect of an aqueous ammonia treatment of AFB1-containing corn on toxicity of corn administered orally to male Fischer rats. Corn was contaminated with AFB1 (5 mg/g corn) and administered per os (2.0 or 4.0 g corn/kg) or after treatment with ammonium hydroxide. Body weight changes, liver weight, hexobarbital sleeping times, hepatic microsomal concentrations of protein and cytochromes P-450 and b5, and mortality were determined 72 hours after dosing with corn, and serum alkaline phosphatase activity was determined 96 hours after dosing. The ammonia treatment of contaminated corn prevented the changes in these parameters caused by AFB₁. Ammoniation of corn free of aflatoxin had no adverse effect on the parameters. although ammoniation, per se, did raise the concentrations of hepatic microsomal protein. The results of the study indicated that ammoniation may prevent acute aflatoxicosis produced by aflatoxin-contaminated corn.

Southern and Clawson (571) conducted studies on the feeding of aflatoxin-contaminated corn to growing rats. Corn was treated with ammonia (Am) according to the NRRC method, and the AFB₁ level was reduced from 2,000 ppb to 510 ppb. Ninety-six male Sprague-Dawley rats (75 g average) were randomly divided into eight treatment groups with 12 rats per treatment. A fortified 12% protein corn-soybean meal diet served as the control (C) diet. The corn used in the C diet contained no detectable aflatoxin. Treatment 2 contained 1,670 ppb aflatoxin; treatments 3 and 4 were similar to treatments 1 and 2, respectively, except that the corn used was ammoniated. Treatments 5 through 8 contained 830, 420, 210, and 100 ppb total aflatoxin, respectively. Liver weights expressed as a percentage of final live weight were increased (P < 0.5) in rats consuming diets containing 1,670 ppb aflatoxin compared to other treatments. Serum albumin, IgG, and IgM concentrations were not affected by ammoniation or by the concentration of aflatoxin in the corn. At the end of 4 weeks, total serum protein had increased (P < 0.5) in rats consuming diets containing aflatoxincontaminated (AFC) corn and Am corn, compared to those consuming the C diet. Average daily gain and average daily feed consumption were reduced (P < 0.01) in rats consuming diets containing 1,670 ppb aflatoxin. The growth of rats consuming AFC corn that had been ammoniated was equal to that of rats consuming a similar concentration of aflatoxin by dilution. From these results and the results of others, it appears that ammoniation of corn contaminated with aflatoxin may be a practical and economical method for detoxification.

In experiments to demonstrate the safety of ammoniated corn, Norred (449) studied the excretion and distribution of ammoniated ¹⁴C- labelled AFB₁-contaminated corn. Finely ground corn to which "C-AFB1 was added (0.5 \(\mu \)Ci/g) was treated with ammonia or not treated, and administered to male Fischer rats (0.1 g corn per rat, intragastrically). Rats, which received nonammoniated corn (NAC), excreted less radioactivity (67 percent of dose) over a 72 hour period than those given ammoniated corn (AC) (78 percent of dose). Radioactivity distributed in the liver of NAC-treated rats reached a peak of 13 percent of the dose 3 hours after administration, and declined to 4 percent of the dose in 72 hours. Ammoniation decreased the binding of labelled compounds to liver. AC-treated rats had < 0.5 percent of the radioactive dose in the liver at three hours and < 0.2 percent by 72 hours. Measurable amounts of radioactivity were not found in blood of AC-treated rats at any time from 1 to 72 hours after administration. Blood levels of labelled compounds of NAC-treated rats peaked at 6 percent of the dose 3 hours after treatment and were still 1 percent of the dose after 72 hours. After feeding rats with NAC, 10 percent of the dose of radioactivity was excreted in urine within 72 hours and 8 percent after treatment with AC. The results indicate that products resulting from ammoniation of aflatoxin-contaminated corn are

poorly absorbed from the gastrointestinal tract, rapidly excreted in urine, and poorly bound to liver.

Large-scale feeding studies to further evaluate the safety of ammonia-decontaminated corn were initiated by USDA in 1975 at the recommendation of the Food and Drug Administration. The protocol for the evaluation consisted of two phases. First, swine, laying hens, and beef cattle were fed either aflatoxin-free corn, ammoniated aflatoxin-free corn, aflatoxin-contaminated corn containing 1,000 ppb total aflatoxins, or ammoniated aflatoxincontaminated corn. Then, meat and egg tissues obtained from these livestock were fed to Fischer 344 rats in both chronic experiments and reproductive studies. Of the parameters measured during the livestock feeding phase, few differences were noted in the laying hens or beef cattle that could be attributed to the dietary treatments. However, in the swine study, there was an apparent reduction in average daily gain due to aflatoxin-contaminated corn. A similar decrease in feed consumption was noted. Ammonia treatment appeared to improve daily gain and feed consumption, but the effect was not significant at the 0.05 level. The results, however, indicated that the positive control, i.e., the AC diet, did increase liver weight, a well-known indicator of aflatoxicosis. This effect was prevented by ammoniation of the corn.

The second phase of the protocol, in which the tissues were fed to rats, has been completed; however, these data have not been summarized nor has the histopathology been completed. Examination of the available data, including body weights, food consumption, reproductive studies, and gross pathology, has not revealed any deleterious effects associated with ammonia treatment (448).

The studies undertaken thus far provide strong evidence that atmospheric ammoniation of corn is a safe and effective method for detoxifying corn that otherwise could not be utilized for livestock feeding. The process represents a cost-effective method for relieving a severe economic problem faced by grain producers. It must be emphasized, however, that approval for ammoniation of aflatoxin-contaminated corn has not been given by FDA, and it is likely that other safety studies may be required before either FDA or USDA can recommend the use of the process.

Nofsinger and Anderson (446) stored aflatoxin-contaminated corn treated with 1 and 1.5% ammonia at ambient temperatures through a Midwestern winter. Average temperatures over the six-month period varied from -5°C to 16°C. The three lots, with initial AFB₁ contents of 896, 461, and 226 ppb, had final AFB₁ levels of 103, 27, and 15 ppb, respectively, for the 1% ammonia treatment, and 32, 8, and 2 ppb, respectively, for the 1.5% treatment.

Doyle and Marth (176, 177) have reported that bisulfite reacted with purified AFB₁ and AFG₁ in a potassium acid phthalate-NaOH buffer (pH 5.5) containing 1.3% methanol, with the result that fluoresence was lost. Reaction rates were influenced by temperature as well as bisulfite concentration. They also found that citric acid and excessive methanol retarded aflatoxin degradation. In other studies, Moerck et al. (423) used sodium bisulfite, sodium hydroxide, and aqueous ammonia as agents for treating corn naturally contaminated with aflatoxin. Corn containing 235 ppb of AFB₁ and AFB₂ were adjusted to a moisture content of 20 percent and then treated for 24 hours at ambient temperature with NaHSO₃, NaOH, or aqueous NH₃ at 0.5 percent, 1.0 percent, or 2.0 percent concentrations. All treatments were effective in reducing aflatoxin B₁ and B₂ levels. Sodium bisulfite was more effective in destroying aflatoxins than were NaOH or aqueous NH3 at 0.5 percent and 1.0 percent concentrations, whereas NaOH and aqueous NH3 were more effective than bisulfite at 2.0 percent concentration. Subjecting yellow corn samples to either NaHSO3,

NaOH, or aqueous NH $_3$ at 2.0% concentrations reduced AFB $_1$ and AFB $_2$ levels to below the FDA guideline of 20 ppb total aflatoxin. Sodium bisulfite was also effective in reducing the levels of aflatoxins in a white dent corn sample containing 81 ppb of aflatoxin B $_1$ and 12 ppb of aflatoxin B $_2$. These results suggest that NaHSO $_3$, NaOH, or aqueous NH $_3$ can be used effectively to destroy aflatoxins in corn and, possibly, in other agricultural commodities.

Liuzzo and Ochomogo (375) described studies in which various concentrations of hydrogen peroxide, ammonium hydroxide, formaldehyde, sodium hypochlorite, and isopropyl alcohol were applied to corn and peanuts contaminated with aflatoxin. Results showed highly significant differences between treatments (P < 0.01). TLC analyses indicated that 1.5 percent hydrogen peroxide, 1.0 percent sodium hypochlorite, and 75.0 percent isopropyl alcohol significantly reduced aflatoxin contamination to nondetectable levels. The latter is not economically feasible due to high cost and the added equipment necessary for its recovery. Formaldehyde (2.0 percent) reduced the concentration to 10 ppb, which is less than the amount of aflatoxin permitted by the FDA in animal feeds. The results of this study suggested that hydrogen peroxide or sodium hypochlorite at concentrations varying from 1.0 to 2.0 percent (w/v) are most efficient considering detoxification level, cost, and availability.

Castegnaro et al. (117) have shown that the use of sodium hypochlorite solution over a long period as a reagent for treating laboratory wastes and equipment contaminated with AFB₁ can lead to the formation of aflatoxin B₁-2,3,dichloride, a known carcinogen and mutagen. If, before disposal, the treated solutions are diluted to 1-1.5 percent (by volume with respect to hypochlorite), followed by the addition of acetone to give a final concentration of approximately 5 percent (v/v), this carcinogen is eliminated.

Elahi and Draughon (193) reported that insecticides registered for use on corn can inhibit AFB₁ production. The insecticides toxaphene, carbaryl, Dasanit, Bux, carbofuran, Dyfonate, EPN, and heptachlor were tested for their potential to inhibit aflatoxin production and growth by A. flavus in YES broth; identification and quantitation of AFB₁ was performed by HPLC. Toxin production was inhibited by Bux (80 percent), toxaphene (82 percent), carbaryl (51 percent), and Dyfonate (60 percent), but heptachlor and EPN did not significantly affect toxin production. Bux and carbofuran both inhibited growth by 40 percent; however, carbofuran did not affect toxin production.

Chakrabarti (118) found that 3 percent hydrogen peroxide, 75 percent methanol, 3 percent perchloric acid, or 5 percent dimethylamine hydrochloride could reduce the AFB $_1$ from 397 ppb in contaminated corn to a level of 20 ppb or less. The biggest drawbacks for use of these alternative chemicals were the apparent necessity to grind the corn before treatment and the high cost of the chemicals.

It appears that, to date, the use of ammonia for detoxifying aflatoxin corn presents the only feasible approach. A process has been reduced to practice for conducting decontamination of such corn on a farm scale and offers a viable option for salvaging otherwise worthless materials. Feeding studies indicate that aflatoxin-contaminated corn treated by the ammonia process has had no adverse effects on cattle, swine, or chickens. These studies continue to provide the necessary pathological and histological data required to obtain FDA approval for the process. Studies conducted since publication of the above process have attempted to use alternative chemicals for inactivation or removal of aflatoxin in corn. Although some success has been achieved in reducing aflatoxin in the laboratory, none of the procedures have been attempted on a practical scale.